

**IN THE SPECIFICATION**

After the Title on page 1, and before the first full paragraph beginning at line 5, please insert the following paragraph:

--This patent application is a continuation of application Ser. No. 08/828,415 filed March 28, 1997, now allowed.--

Replacement for second full paragraph at page 3, lines 13-31:

The constitutive expression by fibrocytes of the surface proteins known to be necessary for antigen presentation contrasts with what has been described for tissue fibroblasts which require activation by interferon- $\gamma$  to express measurable quantities of HLA-DR (Geppert and Lipsky, *J. Immunol.* 135:3750-3762, 1985). Although several tissue-derived cells have been shown to be capable of presenting antigen to memory T cells, including dermal fibroblasts, endothelial cells, and melanocytes (Pober et al., *J. Exp. Med.*, 157:1339-1353, 1983; and Poole et al., Le Poole et al., J. Immunol. [[12]] 151:12,7284-7292, 1993), sensitization of native T cells has been considered to be a particular function of dendritic cells (Inaba et al., *J. Exp. Med.* 172:631-640, 1990; and Levin et al., *J. Immunol.* [[12]] 151:12,6742-6750, 1993). Fibrocytes also present antigen to naive T cells but are distinct from dendritic cells and their precursors not only in their growth properties (fibrocytes are an adherent, proliferating cell population whereas dendritic cells are non-adhering and poorly proliferating) but also in their surface protein expression (collagen $^+$ /CD13 $^+$ /CD34 $^+$ /CD25 $^-$ /CD10 $^-$ /CD38 $^-$ ).

Replacement for second full paragraph at page 13, lines 10-33:

The fibrocytes were examined for their capacity to present soluble antigen in an autologous, T cell proliferation assay. T cells were purified from the peripheral blood of tetanus toxoid-immunized individuals and stimulated with tetanus toxoid *in vitro* together with fibrocytes

as APCs. To assess the functional capacity of fibrocytes to present antigen, we examined the ability of purified human fibrocytes to activate allogeneic T cells in a mixed leukocyte reaction. Three human blood donors were boosted intra-muscularly with 4 units of tetanus toxoid (Connaught Laboratories, Swiftwater, PA). One month later, the PBMCs were isolated from the peripheral blood. T cells were isolated by high-affinity negative selection (human T Cell Enrichment Column, R&D Systems, Minneapolis, MN). FACS analysis of anti-CD3 (Pharmingen) labeled cells indicated that 85-95% of all cells recovered were CD3<sup>+</sup>. APCs were prepared as described above and treated with 25 mg/ml mitomycin C (Stigma) in RPMI medium containing 10% human AB serum (RPMI/10% HS) for 30 minutes and then washed 5 times with RPMI/10% HS. For each assay, the T cells ( $2 \times 10^5$ ) were incubated with mitomycin C-treated autologous fibrocytes, monocytes, or dendritic cells at various T cell:APC ratios in the presence of 2 mg/ml tetanus toxoid in RPMI/10% HS (Jasmes James, in *Current Protocols in Immunology* Coligan et al. eds. John Wiley and Sons, Inc. New York pp. 7.10.1-7.11.4, 1991). After 4, 5 and 6 days of co-culture, the proliferative activity was measured over 12 hours by the incorporation of [<sup>3</sup>H]thymidine (4 mCi/ml) into DNA as measured by liquid scintillation counting. Controls that were included in each experiment were APCs alone, T cells alone, APCs + tetanus toxoid, T cells + tetanus toxoid, and APC + T cells. Mixed leukocyte reactions were conducted similarly except that the fibrocytes and T cells were isolated from allogenic donors and no antigen was included in the co-culture Jasmes (James 1991, *infra.*) Statistical significance as assessed by two sample T-tests (assuming unequal variances) (Zar, in *Biostatistical Analysis*, Prentice Hall, Engelwood Cliffs, New Jersey, [[p718]] pp. 176-179 , 1984).

Replacement for third full paragraph at page 15, line 27 through page 16, line 13:

Although several cell types have been shown to be capable of presenting antigen to memory T cells, the priming of naive T cells has been considered to be a specialized function of "professional" APCs, particularly dendritic cells (Jasmes James, 1991 *infra.*; Levin et al., *J. Immunol.* 12:6742-6750, 1993). To test the ability of mouse fibrocytes to prime naive lymph node T cells *in vivo*, we pulsed mouse fibrocytes with p24 or gp120 *in vitro* and injected them intra-dermally into the rear foot pad of unprimed BALB/c mice. Five days later, the popliteal lymph nodes were removed and the constituent cells tested for a re-stimulation proliferative response. Purified BALB/c fibrocytes were cultured for 3 days with 50 mg/ml of p24 or gp120 in DME/20% FCS, washed 5X in PBS, and injected intra-dermally ( $5 \times 10^4$  cells in 20 ml PBS) into the right rear footpad. The proximal popliteal lymph nodes were explanted 5 days later and cell suspensions prepared by teasing with fine forceps.  $2 \times 10^5$  lymph node cells/well were cultured with 50 mg/ml of antigen in Click's medium (Gibco) supplemented with 1% heat-inactivated mouse serum (Sigma) and 50 mM 2-mercaptoethanol (Sigma) for 72 hours. The proliferative activity was measured over the last 12 hours of culture by the incorporation of [<sup>3</sup>H]thymidine (4 mCi/ml) into DNA. Proliferating cells were identified to be primarily CD4<sup>+</sup> T cells through depletion of CD4<sup>+</sup> T cells by immunomagnetic selection (Dynabeads M-450 L3T4, CD4, Dynal) just prior to liquid scintillation counting. In certain experiments, DBA-2 x C3H/HeJ F<sub>1</sub> mice (H-2<sup>dxk</sup>) were injected with pulsed fibrocytes from either parent strain (DBA-2, H-2<sup>d</sup> or C3H/HeJ, H-2<sup>k</sup>), and 5 days later, the proximal popliteal lymph nodes were isolated and depleted of endogenous class II MHC<sup>+</sup> APCs by immunomagnetic selection (Dynabeads M-450 sheep anti-rat IgG, Dynal, anti-murine class II MHC, rat IgG<sub>2b</sub>, clon ER-TR 3, Accurate).  $1 \times 10^5$  APC-depleted lymph node cells then were co-cultured with  $1 \times 10^5$  mitomycin C-treated F<sub>1</sub> or parent

spleen cells as APC with or without gp120 for 72 hours. The proliferative activity was measured as described above.

Replacement for second full paragraph at page 16, line 20 through page 17, line 2:

This example illustrates antigen-pulsed fibrocytes were not simply transferring antigen to other host APC types. This example provides the results of experiments in which antigen-pulsed fibrocytes from two parent mouse strains were injected into F<sub>1</sub> offspring mice. The T cell reactivity of F<sub>1</sub> offspring was confined predominantly to antigens presented by one of the parental strains, and the priming and re-stimulation APCs must necessarily share the same haplotype (Inaba et al., *J. Exp. Med.* 172:631-640, 1990; Sprent, *J. Exp. Med.* 147:[1159] 1142-1158, 1978; Sprent, *J. Exp. Med.* 147:1142, 1978). DBA-2 x C3H/HeJ F<sub>1</sub> mice (H-2<sup>dxk</sup>) were injected with pulsed fibrocytes from either parent (DBA-2, H-2<sup>d</sup> or C3H/HeJ, H-2<sup>k</sup>) and, five days later, the proximal popliteal lymph nodes were isolated and depleted of endogenous class II MHC<sup>+</sup> APCs by immunomagnetic selection. The F<sub>1</sub> APC-depleted lymph node cells were cultured with F<sub>1</sub> (H-2<sup>dxk</sup>) or parent strain (H-2<sup>d</sup> or H-2<sup>k</sup>) spleen cells as the source of APC, with or without gp120. As shown in Figure 10, the F<sub>1</sub> APC-depleted lymph node cells were reactive to antigen in the presence of the F<sub>1</sub> re-stimulation APCs when priming was performed with fibrocytes from either parental strain. However, if a parental strain was used as the source of re-stimulation APCs, the F<sub>1</sub> APC-depleted lymph node cells would only proliferate if the priming fibrocytes were from the same parental strain. These data indicate that fibrocyte priming and APC re-stimulation of sensitized T cells occurs only in the setting of a shared MHC haplotype. Thus, fibrocytes do not function merely to deliver antigen to other APCs, but rather act to directly sensitize naive T cells in a MHC-specific manner.